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To: Mr. J. L. Charles Date: October 29, 1981

From: T. Yu

Subject: Project Charge Number 6902 (Biochemical Special Investigations)--
Plans and Objectives for 1982

I. OBJECTIVES

a) To investigate the feasibility of new assay systems for the evaluation of cigarette smoke products.

b) To establish and to apply short-term *in vitro* assays to evaluate potential activities of:

1) new cigarette additives, and

2) cigarette smoke products

c) To determine the component(s) responsible for cigarette smoke product activity in each assay.

The yeast mitotic gene conversion assay will be a continuing effort from the former Project 6906. New short-term assays in the areas of bacterial mutagenesis (*Salmonella typhimurium*-forward mutation based on 8-azaguanine resistance) and mammalian cell cytogenetics (sister chromatid exchange) will be added to the project. In addition, a feasibility study of using a plant system (*Tradescantia* stamen hair mutation assay) to evaluate the potential activity of cigarette smoke will also be carried out in 1982.

We are actively recruiting two staff members for Project 6902: a scientist and a research scientist. Both will be experienced individuals who can work independently on the planned assay systems. The research scientist will be involved in the sister chromatid exchange assay. In this regard, the time schedule for this assay will be planned after the research scientist joins the project.

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II. PLANS

A. Yeast Mitotic Gene Conversion Assay

1. Introduction

This assay has been a part of a battery of short-term assays for the past several years. *Saccharomyces cerevisiae* strain $D_4$ has been used as the tester strain. To date, the activity of a variety of samples have been

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determined in this assay system. The results showed that all CSC samples tested generally exhibited a dose-response effect and that the assay is able to distinguish activities among various types of smoke products derived from different cigarettes. One drawback of these results is the fact that the average overall sensitivity of the assay is not very high. For example, the activity of the majority of the samples tested did not show a two-fold increase over that of the background control, which is the generally accepted criterion for a test compound to be active in short-term *in vitro* assays.

2. Plans

1) Strain $D_4$ (Fourth Quarter, 1981 and First Quarter, 1982)

a. Continue to study the cigarette smoke product activity in close collaboration with Project 6908 personnel as in the past. Experiments are currently carried out to test burley: CSC, acid, base, and neutral fractions. The results of these experiments will be used as guidelines to determine the directions of work involved with strain $D_4$. Upon completion of this work, a special seminar will be given by L. H. Thompson to the Manager, Technical Coordinator, and the Project Leaders of the division to make a thorough evaluation of the yeast mitotic gene conversion assay using strain $D_4$. A definitive decision whether to use strain $D_4$ for future work should be reached no later than the first quarter, 1982.

2) Strain $D_7$ (First Quarter, 1982 and continuing)

Strain $D_7$ has been added to our culture collection in 1980. This strain was constructed in a manner that three genetic effects may be monitored simultaneously in the same strain. These effects are: mitotic gene conversion, mitotic recombinations and reverse mutation. Initial experiments will be carried out to establish the $D_7$ assay to monitor mitotic gene conversion with control compounds. Current plans involving work with strain $D_7$ are primarily concerned with testing potential new cigarette additives. Testing of CSC and/or other smoke products would be conducted only as time permits.

B. *S. typhimurium* Forward Mutation Assay Based on 8-Azaguanine Resistance

1. Introduction

The forward mutation assay employs one tester strain, T667. It is designed to measure the ability of a test agent to produce mutant cells in a wild type cell population. The resultant mutant cells are selected by cultivating the treated cell population on growth medium containing 8-azaguanine (8AG), which supports growth of the mutant cells, but not the wild type cells. The assay can be conducted both in the presence and absence of metabolic activation (±S9). Because it is a forward mutation, the target size of the genetic material responsible for the mutational event(s) is larger compared with reverse mutation. Furthermore, it should not exhibit



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mutagen specificity. Theoretically, it should be more, and/or at least as sensitive as the standard *Salmonella*/microsome assay which measures reverse mutational events.

The forward mutation assay has not been widely validated. Literature reports showed that the forward mutation assay measured in one tester strain is equisensitive to the *Salmonella*/microsome assay using five tester strains simultaneously.

The forward mutation assay will be used to test cigarette smoke products and potential new cigarette additives.

2. Plans

Work concerning the forward mutation assay has been planned in three phases:

Phase 1: Assay Establishment and Validation (First Quarter, 1982)

Initially, a visit will be made to the outside laboratory where the assay was developed. Two goals will be accomplished during the visit. (1) To become familiar with all aspects of the mutation assay and to acquire the necessary stock cultures. (2) To learn and to become familiar with the procedures involved in interfacing the microassay version of the forward mutation test to evaluate activity of HPLC fractions, particularly with respect to automation. Subsequent to the visit, the assay will be established at PM using positive and negative control compounds in the presence and absence of metabolic activation (±S9).

After assay establishment, effort will be directed toward building a data base for assay validation. Fifteen to 20 compounds from different classes of chemical will be selected and evaluated for their response in the assay. Preferably, these will be paired compounds consisting of active and inactive counterparts from the same chemical class.

Phase 2: Evaluation of Smoke Products Derived from Selected Cigarettes (Second Quarter and Continuing)

This phase of work will be coordinated closely with Project 6906 and 6908 personnel.

1) Cigarettes selected for investigation will be Kentucky reference cigarette 2R1, burley, a low tar filler and RL. The activity of CSCs and acid, base and neutral fractions derived from these CSCs will be evaluated. Subfractions of the active fractions will be further evaluated. The purpose of these studies will be to establish a data base which can be compared with that from the standard *Salmonella*/microsome assay.

2) Interface forward mutation assay with HPLC fractions. Burley cigarettes will be used for these investigations. Fractions that are active in the forward mutation assay will subsequently

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be tested in *Salmonella*/microsome assay using all five strains. This is done with the attempt to characterize the mutation specificity, *i. e.*, whether the detected activity is due to base-pair substitution or frame-shift mutation. The results generated from these biological studies will be used to guide further work in chemical separation of fractions and/or subfractions.

Phase 3: Automation--to be determined

C. Sister Chromatid Exchange (SCE) Assay

1. Introduction

Cigarette smoke condensates have been reported to induce SCE in test systems containing no exogenous metabolic activating enzymes. In our plans, the SCE assay will be used to evaluate the activity of potential new cigarette additives and cigarette smoke products. In addition, the influence of these substances on cell replication kinetics and mitotic index will also be investigated in selected cases.

2. Plans

SCE assay will be conducted in V79 Chinese hamster lung cells. This cell line was acquired by Project 6904 personnel recently and mutation assays using this cell line are currently being carried out.

Plans for SCE assay include:

1) Assay establishment using positive and negative control compounds both in the presence and in the absence of metabolic activation (±S9).

2) Assay utilization as a part of a battery of short-term assay in routine evaluation of potential new cigarette additives, and, later, cigarette smoke products.

3) Assay validation by evaluating the activity of selected cigarette CSCs and derived fractions. Cigarette selected will be 2R1, burley, a low tar filler and RL.

D. *Tradescantia* Stamen Hair Mutation Assay

This assay system is potentially suitable to study the activity of sidestream smoke. The feasibility for conducting this assay at PM should be investigated. The feasibility study will involve literature search, contact with and visits to outside laboratories and a complete assessment of the assay system.

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SUMMARY FOR PLANS AND OBJECTIVES FOR 1982

| | Assay/Activity | Time |
|---|---|---|
| A. | Yeast Mitotic Gene Conversion Assay | |
| | 1. Strain $D_4$ | 4th Quarter, 1981 and 1st Quarter, 1982 |
| | 2. Strain $D_7$ | 1st Quarter, 1982 and continuing |
| B. | *S. typhimurium* Forward Mutation Based on 8-Azaguanine Resistance | |
| | 1. Assay establishment and validation | 1st Quarter, 1982 |
| | 2. Evaluation of smoke products from selected cigarettes | 2d Quarter and continuing |
| | 3. Automation | To be determined |
| C. | Sister Chromatid Exchange Assay | To be determined |
| | 1. Assay establishment | |
| | 2. Evaluation of potential new cigarette additives | |
| | 3. Assay validation using cigarette smoke condensates and derived fractions | |
| D. | *Tradescantia* Stamen Hair Mutation Assay: A Feasibility Study | |

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